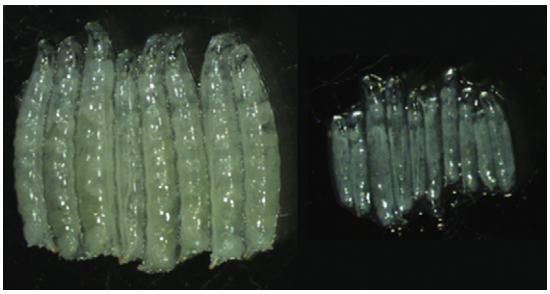
Cell

Microbial Genomics

Outnumbering our own cells by ten to one, the secret lives of our microbiota are beginning to reveal themselves through genomic analyses. This issue's Select features recent studies that begin to clarify how our diminutive consorts can ensure a host's good health but also destroy it.

A Spoonful of Bugs Helps the Sugar Go Down

It is well known that the gut microbiota regulates homeostasis of the digestive system; however, the mechanisms involved have been difficult to uncover. By manipulating the germ environment of *Drosophila* alongside genomic analyses of its symbiont, *Acetobacter pomorum*, Shin et al. (2011) deduce a pathway that relies on the collaboration between a bacterial enzymatic reaction and the host insulin signaling pathway. *Drosophila* play host to five main species of bacteria; completely wiping out the microbiota in germ-free larvae is lethal in a specific-diet condition, with these larvae only reaching 10% of the size of wild-type before they expire. The authors show that recolonization by *A. pomorum* alone is sufficient to restore development and growth rate. To identify mutants that no longer restored growth in recolonized germ-free larvae, Shin et al. constructed a draft genome of *A. pomorum* and screened a newly generated mutant library. This effort uncovered a number of genes that function in the pyrroloquinoline quinone-dependent alcohol dehydrogenase (PQQ-ADH)-dependent oxidative respiratory chain, essential for growth in the host. In animals colonized by bacteria lacking the PQQ-ADH pathway, circulating sugars and lipids are elevated; this phenotype is reminiscent of that for flies with defective insulin/insulin-like growth factor signaling (IIS). The authors go on to show that IIS activation is indeed dependent on the PQQ-ADH pathway, implicating the bacterial production of acetic acid-derived metabolite(s) in insulin signaling and growth-rate regulation. PQQ-ADH activity in *A. pomorum* is essential for normal numbers of intestinal stem cells and epithelial cell renewal rate. This new model to probe interspecies genetic interactions provides an exciting avenue for research into entwined mechanisms of microbe and host signaling pathways.



Germ-free animals colonized with the wild-type *A. pomorum* (left) and the PQQ-ADH mutant lacking acetic acid-producing ability (right). Image courtesy of W.J. Lee.

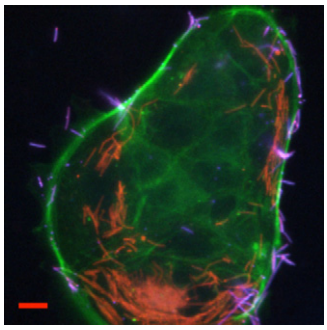
Shin, S.C., et al. (2011). Science 334, 670–674.

Gut Microbiome Enterotypes Hint at Future Diagnostics

The vast majority of the flora of the human gut is made up of a motley crew of bacteria, with low showings from the archaea, viruses, and yeast. Metagenomes of the microbiota from individuals are accruing, though cohort sizes have not been sufficient to draw broad conclusions regarding the community structures of these populations or whether certain compositions correlate with host classifiers such as BMI or gender. Arumugam et al. (2011) now provide valuable insights into the species makeup of gut microbiota, powerfully suggesting that rather than existing as a continuum of different community compositions, individuals exhibit one of three ''enterotypes'' that can be classified. Thirty-nine individuals of several different nationalities (including Europeans, Americans, and Japanese) were sampled, providing robust statistical evidence that the datasets cluster into groups identifiable by variation in one of three genera, *Bacteroides*, *Prevotella*, and *Ruminococcus*. Analysis shows that these three genera strongly correlate (either by co-occurring or avoiding each other), which hints that the enterotypes are driven by groups of species that work together in shaping the community composition. Phylogenetic signals in this cohort did not significantly correlate with biomarkers such as age, nationality, or BMI, but functional biomarkers seemed to provide more robust modules that correlate with hosts' BMI, supporting the suspected link between energy harvest capacity in the gut microbiota and obesity in the host. Unsurprisingly, enterotypes are not sharply delineated like human blood types, but their existence still provides hope that in the future, functional modules (that need to be refined with larger cohort sizes) may be used as diagnostics for some cancers or prognostic markers for changes in the health status of individuals.

Arumugam, M., et al. (2011). Nature 473, 174–180.

Fusobacterium Found within Colon Cancer Cells



F. nucleatum-infected Caco-2 cells, stained for actin (green) and differentially stained for invasive (orange) versus noninvasive (purple) bacteria. Bar = 13 μm. Image courtesy of E. Allen-Vercoe and J. Strauss.

Adding weight to the importance of analyzing the gut microbiota to assess cancer risk, two studies from Castellarin et al. (2011) and Kostic et al. (2011) make the remarkable discovery that an invasive bacterium normally associated with oral inflammation resides within colon cancer cells. Inflammation is a known risk factor for colorectal cancer, so the contribution of inflammatory microorganisms was assessed by these groups with whole-genome sequencing of colorectal cancers and matched normal colons, followed by computational analysis to siphon out the suspected microbial genomes. *Fusobacterium* species are shown to be significantly enriched in colon cancer samples compared to normal tissues, with concurrent depletion of *Bacteroidetes* and *Firmicutes* phyla. This altered community structure in the tumor tissue is suggested by Kostic et al. to be in line with the recently posited "alpha-bug" hypothesis, in which certain microbes with virulence or procarcinogenic capabilities may be able to remodel a community towards proinflammatory conditions and contribute to the transformation of host cells. Histological analyses of surgically resected tissues showed *Fusobacterium* residing in the tumor mucosa and also inside some of the tumor cells themselves. Whether the *Fusobacterium* colonization of the tumor microenvironment represents an opportunistic infection or a contributory inflammatory trigger to tumorigenesis itself has yet to be tackled, but nonetheless this evidence of the close interplay between microbial niches and the tumor environment is intriguing. The suspected contribution of microbial invasion in triggering or enhancing malignancy is corroborated by the finding of Castellarin et al. that lymph node metastases are significantly more prevalent in colon cancer patients showing enrichment of *Fusobacterium*.

Kostic, A.D., et al. (2011). Genome Res. Published online October 18, 2011. 10.1101/gr.126573.111.
Castellarin, M., et al. (2011). Genome Res. Published online October 18, 2011. 10.1101/gr.126516.111.

A jaw bone from which teeth were used to harvest 14th century *Y. pestis* genetic material. Image courtesy of S. De Witte. ©Museum of London.

Black Death Genome

A draft genome of the *Yersinia pestis* strain that caused the bubonic plague outbreak in Europe in the 14th century has been sequenced by Bos et al. (2011) from the skeletal remains of plague victims exhumed in east London. This genome builds on work published earlier this year by the same group of researchers, in which Schuenemann et al. (2011) sequenced the key virulence-associated plasmid pPCP1. This impressive technological feat (not least the recovery of infected teeth at the Museum of London) used DNA capture and enrichment coupled with high-throughput DNA sequencing. When the sequence is compared with the genomes of all currently available strains, the root confirms that the 1347–1351 pandemic was the originating strain for all contemporary *Y. pestis* strains commonly associated with human infections. The sixth to eighth century outbreak, the Plague of Justinian, has been thought to have resulted from the same pathogen, but the Black Death genome sequence calls this theory into question. Newly calibrated tree topologies suggest that all currently circulating *Y. pestis* strains that are pathogenic to humans emerged in the 13th century at the earliest, and the Plague of Justinian seems to be totally different—perhaps caused by a different pathogen altogether. The *Y. pestis* genome reveals a surprising conservation of sequence between ancient and modern plague genomes over the past 660 years but also provides evidence of microevolution of the strain within a single individual. The sequence conservation suggests that existing relatives of the Black Death bacterium contain similar virulence-associated genes, meaning that perceived changes in the virulence and severity of this pathogen are likely to be due to factors other than genetic mutation, including the genetics of the host organisms and synergistic interactions with other diseases. This insight into the evolution of *Y. pestis* virulence will hopefully improve efforts into understanding population susceptibility to emerging and re-emerging pathogens.

Bos, K.I., et al. (2011). Nature 478, 506–510.
Schuenemann, V.J., et al. (2011). Proc. Natl. Acad. Sci. USA 108, E746–E752.

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